support for the new claims is provided in the table below for the Examiner's convenience. Applicants respectfully submit that the specification reasonably conveys that the applicants had possession of the claimed subject matter. M.P.E.P. § 2163.02. Therefore, the specification satisfies the written description requirement regarding the new claims.

New Claim	Support for the new claim in the specification
33	Page 11, lines 9-25; page 14, line 17 to page 15, line 8; page 47, lines 11-12; page 48, line 1 to page 50, line 5; original claims 1, 16 and 23.
34	Page 11, lines 9-25; page 44, lines 10-12; page 48, line 1 to page 50, line 5; page 45, lines 10-20.
35	Page 43, lines 8-16.
36 and 37	Original claims 4, 16 and 18.
38	Original claim 19.
39	Original claim 17.
40	Page 47, lines 11-31; original claim 17.
41	Original claim 21; page 47, lines 11-12.
42	Original claim 3.
43	Original claim 2.
44	Page 36, line 24 to page 38, line 19; original claim 2.
45	Page 45, line 10-20.
46	Page 10, lines 5-12.
47	Page 10, lines 5-23; original claim 27.
48	Page 10, lines 20-31; original claim 4.
49	Page 10, lines 20-31; page 40, lines 11-13.
50	Page 48, line 30 to page 49, line14; original claim 1.
51	Original claim 5; page 18, lines 1-4.
52	Original claim 5; page 18, lines 1-4.

53 and 54	Page 11, lines 9-25; original claim 9.

Applicants respectfully submit that the newly added claims (33-54) replace claims 1-4 and 28-30 to more clearly define the invention as it relates to test cells that express a labeled protein that associates with double minute chromosomes or extrachromosomal DNA. No other aspects of the previously pending claims have been amended and are therefore entitled to a full range of equivalents.

Rejections Under 35 U.S.C. § 112, second paragraph

The Examiner rejected previously pending claims 28 and 30 under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicants regard as the invention. This rejection is respectfully traversed. Claims 28 and 30 have been replaced by new claim 49. Applicants respectfully submit that the inclusion of "further comprising" in claim 49 obviates the Examiner's rejection of previously pending claims 28 and 30. Accordingly, withdrawal of the rejection is proper and is respectfully requested.

Rejections Under 35 U.S.C. § 103

The Examiner rejected previously pending claims 1, 3, 28, 29 and 30 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Eckhardt et al. (Eckhardt et al., Proc. Natl. Acad. Sci. (USA), 91: 6674-6678 (1994)) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci. (USA), 80: 7533-7537 (1983)). This rejection is respectfully traversed with respect to the newly added claims.

New claim 33 relates to a method to identify an agent that increases or decreases the amount of double minute chromosomes or extrachromosomal DNA in a cell, comprising contacting the cell with the agent, wherein the cell expresses a labeled protein that associates with double minute chromosomes or extracellular DNA to form a labeled complex; and comparing the amount of the labeled complex contained in the cell contacted with the agent with the amount of labeled complex contained in a cell that was not contacted with the agent.

New claim 50 relates to a method to identify an agent that increases or decreases the amount of an extrachromosomal DNA in a cell, comprising contacting the cell with the agent, wherein the cell expresses a labeled protein that is a non-centromere binding protein or a lac operator that associates with the extrachromosomal DNA to form a labeled complex; and comparing the amount of the labeled complex contained in the cell contacted with the agent with the amount of labeled complex contained in a cell that was not contacted with the agent.

Eckhardt disclosed that hydroxyurea (HU) can eliminate amplified copies of c-myc located on double minute chromosomes (DMs) which can lead to a reduction in tumorigenicity in vitro and in vivo (abstract). To determine the c-myc copy number, Eckhardt prepared genomic DNA using SDS lysis and determined the c-myc copy number by dot-blot hybridization to a c-myc probe (page 6675). Hybridization with a c-myc probe was also used to quantitate c-myc expression (page 6675). Furthermore, cells were evaluated for micronuclei and DMs by fixing them in methanol and glacial acetic acid followed by counting micronuclei and DMs from preparations of metaphase chromosome spreads and interphase nuclei (page 6675). In situ hybridization with a c-myc probe was also used to determine the presence of a c-myc gene in micronuclei (page 6677).

Snapka disclosed that the rate of loss of the unstably amplified DHFR genes could be greatly increased by growing the cells in the presence of a nonlethal concentration of hydroxyurea (abstract). The dosage of the DHFR gene was determined by DNA-DNA dot-blot hybridization (page 7534, right column; legends of Fig. 1 and Fig. 3). Snapka did not use any method to directly detect or visualize an extrachromosomal DNA, such as a double minute chromosome.

Neither Eckhardt or Snapka, alone or in combination, describe or suggest a method to identify an agent that increases or decreases the amount of double minute chromosomes or extrachromosomal DNA in a cell which expresses a labeled protein that associates with double minute chromosomes or extrachromosomal DNA. Rather, Eckardt and Snapka disclose the use of metaphase chromosome spreads and nucleic acid hybridization methods to visualize extrachromosomal DNA and to determine gene copy number.

Because the references alone or in combination do not teach or suggest the claimed

invention, it is respectfully requested that the Examiner withdraw the rejections under 35 U.S.C. § 103.

The Examiner rejected previously pending claims 1, 3, 4, 28, 29 and 30 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Eckhardt et al. (Eckhardt et al., Proc. Natl. Acad. Sci. (USA), 91: 6674-6678 (1994)) in view of Snapka et al., (Snapka et al., Proc. Natl. Acad., Sci. (USA), 80: 7533-7537 (1983)) and further in view of Dertinger et al. (U.S. Patent No. 5,858,667, published January 12, 1999; filed September 6, 1996). This rejection is respectfully traversed with respect to the new claims.

Snapka and Eckhardt were described previously.

Dertinger describes a single-laser flow cytometric method for determining whether a compound produces micronucleation in erythrocyte populations (Example 5). The method involves treating fixed reticulocytes with RNase and a fluorescently labeled antibody, such as an FITC-labeled antibody, having binding specificity for a surface marker for erythroblasts/reticulocytes (column 9, lines 27-31). The erythrocytes are then stained with a nucleic acid stain, such as the chemical propidium iodide, which stains DNA representing micronuclei (abstract; column 9, lines 27-31). The dual labeled cells are then excited with laser light and light emission is used to determine the presence of micronuclei in the erythrocytes. Thus, Dertinger discloses the use of exogenous methods for staining extrachromosomal DNA with chemical dyes as opposed to Applicants use of a labeled protein to label DNA.

Neither Eckhardt, Snapka, or Dertinger, alone or in combination, describe or suggest a method to identify an agent that increases or decreases the amount of double minute chromosomes or extrachromosomal DNA in a cell which expresses a labeled protein that associates with double minute chromosomes or extrachromosomal DNA.

Because the references alone or in combination do not teach or suggest the claimed invention, it is respectfully requested that the Examiner withdraw the rejections under 35 U.S.C. § 103.

The Examiner rejected previously pending claims 1, 23, 28, 29 and 30 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Eckhardt et al. (Eckhardt et al., Proc. Natl. Acad. Sci. (USA), 91: 6674-6678 (1994)) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci.

(USA), 80: 7533-7537 (1983)) and further in view of Livingstone et al. (Livingstone et al., Cell, 70: 923-935 (1992)). This rejection is respectfully traversed with respect to the new claims.

Eckhardt and Snapka were described previously.

Livingstone examined whether the mutation or loss of one or both p53 alleles was sufficient to allow the amplification of CAD (trifunctional enzyme carbamoyl-P synthetase, aspartate transcarbamylase, dihydroorotase) to occur (page 923). Fluorescence in situ hybridization (Fig. 2) and southern hybridization analysis (page 933) were conducted with a labeled CAD fragment to determine the CAD gene copy number and the sites of the CAD genes in the cell.

Neither Eckhardt, Snapka, or Livingstone, alone or in combination, describe or suggest a method to identify an agent that increases or decreases the amount of double minute chromosomes or extrachromosomal DNA in a cell which expresses a labeled protein that associates with double minute chromosomes or extrachromosomal DNA. Rather, Eckardt, Snapka, and Livingstone disclose the use of metaphase chromosome spreads and hybridization methods to visualize extrachromosomal DNA and to quantitate gene copy number.

Because the references alone or in combination do not teach or suggest the claimed invention, it is respectfully requested that the Examiner withdraw the rejections under 35 U.S.C. § 103.

The Examiner rejected previously pending claims 1, 3, 4 and 29 under 35 U.S.C. § 103(a) as allegedly being unpatentable over Shimizu et al. (Shimizu et al., Nature Genetics, 12:65-71 (1996)) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci. (USA), 80: 7533-7537 (1983)). This rejection is respectfully traversed with respect to the new claims.

Snapka was described above.

Shimizu describes a method for purifying double minute chromosomes (DMs) from micronuclei isolated from a cell (abstract). The method involves the use of hydroxyurea (HU) to induce micronuclei formation and then separation of double minute chromosomes from the micronuclei. These double minute chromosomes can then be used to generate nucleic acid probes for use in *in situ* hybridization to determine gene dosage and the chromosomal location that generated the double minute chromosomes (page 67 and Figure 2). Shimizu also described

the use of the chemical dye, 4,6-diamino-2-phenylindole (DAPI), to stain DNA in micronuclei preparations (page 66).

Neither Snapka, or Shimizu, alone or in combination, describe or suggest a method to identify an agent that increases or decreases the amount of double minute chromosomes or extrachromosomal DNA in a cell which expresses a labeled protein that associates with double minute chromosomes or extrachromosomal DNA. Rather, Shimizu discloses the use of a chemical dye to stain DNA and nucleic acid hybridization to localize a gene (page 66 and figure 1). Snapka discloses the use of nucleic acid hybridization methods to quantitate gene copy number.

Because the references alone or in combination do not teach or suggest the claimed invention, it is respectfully requested that the Examiner withdraw the rejections under 35 U.S.C. § 103.

The Examiner rejected previously pending claims 1, 3, 4 and 29 under 35 U.S.C. § 103(a) as allegedly being unpatentable over U.S. Patent No. 6,033,849 (Wahl et al.) in view of Snapka et al. (Snapka et al., Proc. Natl. Acad. Sci. (USA), 80: 7533-7537 (1983)). This rejection is respectfully traversed with respect to the new claims.

Snapka has been described previously.

Wahl described a method for isolating and identifying an extrachromosomal amplified target nucleic acid from micronuclei in a cell. The method involves contacting a cell suspected of having extrachromosomal amplified target nucleic acid with a non-alkaloid agent capable of inducing the formation of micronuclei in the cell, isolating the micronuclei from the cell, isolating the extrachromosomal target nucleic acid from the isolated micronuclei and hybridizing the nucleic acid from the isolated micronuclei with chromosomal DNA from the cell, wherein the isolated micronuclei nucleic acid is a probe, and identifying the amplified target nucleic acid (Figure 1). Micronuclei were visualized by preparing metaphase chromosome spreads (column 10, lines 10-12).

Neither Snapka, or Wahl, alone or in combination, describe or suggest a method to identify an agent that increases or decreases the amount of double minute chromosomes or extrachromosomal DNA in a cell which expresses a labeled protein that associates with double

minute chromosomes or extrachromosomal DNA. Rather, Snapka discloses the use of nucleic acid hybridization methods and Wahl discloses nucleic acid hybridization and metaphase chromosome spreads to identify target nucleic acid (column 10, lines 11-28).

Because the references alone or in combination do not teach or suggest the claimed invention, it is respectfully requested that the Examiner withdraw the rejections under 35 U.S.C. § 103.

Conclusion

Applicant respectfully submits that the claims are in condition for allowance and notification to that effect is earnestly requested. The Examiner is invited to telephone Applicant's attorney (612/371-2123) to facilitate prosecution of this application.

If necessary, please charge any additional fees or credit overpayment to Deposit Account No. 19-0743.

Respectfully submitted,

GEOFFREY M. WAHL ET AL.

By their Representatives,

SCHWEGMAN, LUNDBERG, WOESSNER & KLUTH, P.A.
P.O. Box 2938
Minneapolis, MN 55402
(612) 373-6939

Date 4-23-02 By _____
Leif T. Stordal
Reg. No. 46,251

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